"Biological Synchronization of The Endo-parasitoid *Platygaster demades* Walker (Hymenoptera:Platygasteridae) With Its host The Olive Leaf Midge *Dasineura oleae* F. Loew (Diptera: Cecidomyiidae)"

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ABSTRACT

The biology of the Olive Leaf Midge (OLM), *Dasineura oleae* (Diptera:Cecidomyiidae) F. Loew and the endo-parasitoid *Platygaster demades* (Hymenoptera : Platygasteridae) Walker was studied on olive orchards in the Syrian Coast in Lattakia province during 2013 & 2014. Presence of one generation of the olive leaf midge on the leaves was registered. Biological synchronization of the endo-parasitoid *P. demades* with that of its host was noticed. No adults of the endo-parasitoid were registered except for the host activity period. Percent appearance of the endo-parasitoid reached up to 77.9 & 65.5% for 2013 & 2014 in April, respectively. During the study, the percent of endo-parasitoid diapause reached 100% during the host diapause period. The dissection of host larvae has proved that there were endo-parasitoid 's larvae inside the second larval stage of the host larvae at diapause stage, lasting for early activity of the host. This relationship between endo-parasitoid 's larvae & host's larvae during the activity period was clear. The development of olive leaf midge's population and the endo-parasitoid was studied per month in samples under field conditions.

Key words: Olive Leaf Midge, *Dasineura oleae* , Synchronization ,Syrian Coast

1. INTRODUCTION

Synchronization of endo-parasitoid lifecycle with that of their host insect may be found in insect kingdom, particularity for those which have a diapause phase during their biological life or have a few generations i.e. 1 to 2 generation per year. This case can be seen on olive leaf midge under study.

The OLM *D. oleae* F. Loew prevails in the Mediterranean area. Severe infections on olive trees in the coastal area and Greek have been registered. Over the recent years, its prevalence was noticed on olive orchards in different areas of the Syrian coast, severely damaging leaves and inflorescences (Mustafa, 1990; Patil *et al.*,1998; Baidaq, 2010; Karataraki *et al*., 2010). And Hrncic (1998) has mentioned that OLM is an important insect on olives in Black Mont.

The last studies showed that there were some parasites which fairly could reduce the attacks of OLM. The family Platygastridae is the most important one which contains many parasite species, the most important of which *Platygaster* spp. Some of these species are *P. apicalis* Thomas, *P. mayetiolae* Kief, and *P. oleae* Szeleny, as well as some species of other genus *Synopeas figitiformis* Thomas, *Quadrastichus dasineurae* (Karataraki *et al.*,2010 cited from Doanlar *et al.*, 2008).

In addition to *Platygaster demades* Walker we registered, for the first time in Syria, that is an endo-parasitoid on olive leaf midge larvae. This species is known worldwide that is used to control the pear leaf rolling midge *Dasineura pyri* in New Zealand. Now, the endo-parasitoid *P.demades* is the most important natural enemy in New Zealand (Tomkins *et al.*, 2000). This endo-parasitoid was also registered as an egg parasite on apple leaf curling midge *Dasineura mali* in Europe, reducing the infection of the apple leaf curling midge *D. mali* (Shaw *et al*., 2005 cited from Trapman 1988; Carl,1980).

This research aims, for the first time, at studying the biological synchronization of OLM *D. oleae* with that of *P. demade*s. Due to lack of studies on this endo-parasitoid on *D. oleae* and its importance as an endo-parasitoid on OLM in the Syrian coast, it was essential to identify its biology.

2. MATERIALS & METHODS

2.1. Sampling

Samples from different olive orchards on an area of 7-8 ha in Lattakia, 250-350 m above sea level were collected. The samples were randomly and weekly collected in 2013 and 2014 from infected trees with galls on leaves & inflorescences. Each sample has 20 branches, taken randomly from the four directions and the middle of the tree. Each branch has 10-12 leaves. The branches were placed in transparent air-emptied plastic bags, firmly closed and taken to the laboratory.

Each 20 branches was put in a transparent plastic box (25 x 15 x 12 cm). The sample was left for a week at laboratory temperature with good light. We counted the numbers of emerging adults, if any, in both the olive leaf midge & the endo-parasitoid *P. demades* , then we count the number of galls in the sample. We examined the remaining galls for registering the different stages of both the midge & its parasitoid.

2.2. Parasitism

2.2.1. Parasitism according to the pupae & adults of the parasitoid:

The number of emerging adults and pupae of the parasitoid within four readings per month was counted. Then, we took the mean parasitism per month during the study period 2013 & 2014 as follows:

$$\text{Parasitism } \% = \frac{\bar{X} \pm SD}{N} \times 100$$

$\bar{X}$: Mean number of adults & pupae of parasitoid per monthly sample.
N : Total number of galls per monthly sample

2.2.2. Parasitism according to OLM larvae's anatomic examination:

We anatomized the Olive leaf midge's larvae during the diapause period from early July to end December for 2013 & 2014. The host inactivity was at the second larval stage. We examined two samples in the 1st & 4th week of the month. Each sample had 50 larvae. We used a microscope 90 X – Optica. We counted the average number of larvae attacked by the parasite and later counted parasitism as follows:

$$\text{Parasitism } \% = \frac{\bar{X} \pm SD}{N} \times 100$$

$\bar{X}$: Mean number of larvae attacked by the parasitoid
N :Total number of larvae (n=50)

2.3. Percent different stages of the midge & the parasitoid

Galls were counted in the weekly samples with four readings per month, and they were examined by microscope 90 X – Optika. We registered the different stages of midge & endo-parasitoid in their population and determined the parasite larvae inside host larvae of the parasitoid's stage; especially during the diapause period from early July to end December in 2013 & 2014. The mean percentage of the different stages for the midge & the parasite in their population per month was counted as follows:

$$Pw = \frac{No}{T} \times 100 \quad , \quad Pm = \frac{\sum Pw}{4}$$

Pw: Percent of the midge's or parasitoid's per stage in the weekly sample
No: Populations of midge or parasitoid per stage
T: Total number of the midge or parasitoid members in the weekly sample
Pm: Mean of Percent of the midge's or parasitoid's per stage in the monthly sample

2.4. Statistical analysis

The data was analyzed by Student t test and the significance among the data was separated at (p.0.05).

3. RESULTS AND DISCUSSION

3.1. Parasitism *of P.demades* on its host during 2013 & 2014

3.1.1. Parasitism, based on the presence of pupae & adults of the endo-parasitoid :

Fig. (1) showed parasitism based on the presence of the endo- parasitoid pupae & adults in the field samples, ranging between 3.14-29%. The pupae & adults were found during the activity period of its host as from mid-January to end June. The highest parasitism was 23.28% in January 2013 versus 29 % in February 2014. In general, parasitism was rather low particularly in May & June i.e. 4.55 & 3.14% in June for 2013 & 2014, respectively. This is due to climate fluctuations where temperature was relatively high during this period.

We registered a parasitism about 3 % in October & November during the study period. The existence of this parasitoid in this period may be due to the appearance of some host larvae which left the diapause & continued their development, in addition to temperature changes in October & November. However, some host larvae attacked by the parasite were present, resulting in continuation of parasitoid development.

Back to studies conducted by Shaw *et al.*(2003) who has shown parasitism for *P. demade*s up to 90% on mature larvae in one generation for its host apple

leaf curling midge *D. mali*. Comparing with other species of the same genus, species *P. oebalu*s & *P. tisia*s have parasitism over 70% on larvae of rape leaf midge *D. brassicae* (Axelsen,1994 ; Buchi & Keller ,1994), while that of species *P. marchali* was 40% on pear leaf midge *D. byri* (Rieux *et al*., 1990).

3.1.2. Parasitism depending on the anatomic examination of the olive leaf midge' s larvae in the second stage:

Table(1) showed the anatomic examination of olive leaf midge' s larvae in the second stage during 2013 and 2014. There were some larvae attacked by the parasitoid . The endo- parasitoid was found in its first larval stage inside the host larvae. Parasitism ranged between 29.5 – 39% during diapause of host larvae. The highest parasitism was 38% & 39% in December and October in 2013 & 2014, respectively.

Table1. Parasitism on the olive leaf midge larvae during the diapause period (N =50).

Month	Year	Damaged larvae $\overline{X} \pm SD$	Undamaged larvae $\overline{X} \pm SD$	Parasitism %
Jul	2013	16 ± 2	34 ± 2	32
	2014	18 ± 4	33 ± 4	35
Aug	2013	16 ± 3	34 ± 3	32
	2014	15 ± 3	35 ± 3	29.5
Sep	2013	16 ± 3	34 ± 3	32
	2014	15 ± 3	35 ± 3	30
Oct	2013	16 ± 2	35 ± 2	31
	2014	20 ± 1	31 ± 1	39
Nov	2013	16 ± 0	34 ± 0	31.5
	2014	15 ± 3	35 ± 3	30
Dec	2013	19 ± 2	31 ± 2	38
	2014	18 ± 3	32 ± 3	35.5

***Means during 2013 & 2014 are not significantly different at 5% level.by Student t test (T_1= 0.16< T_o=1.96)**

The behavior of the endo- parasitoid of OLM may be similar to some species of parasitoid that attack the midges of Cecidomyiidae family. A study of the parasitism of sorghum midge *Contarinia sorghicola* (Coquillett) has showed that its attack by many parasites the most important of which *Tetrastichus venustus.* The parasitism of *T. venustus* was 20% and this percent may be less (Baxendale *et al* .,1983). Parasite larvae remain in diapause as its host larvae are in diapause in October. Parasite larvae became active when their host is under activity in spring (Baxendale & Teetes ,1983). Synchronization between emerging adults of *T. venustus* and the time of emerging adults of sorghum midge *C. sorghicola* was noticed during the activity period of the sorghum midge *C. sorghicola*. However, *T. venustus* was clearly the dominant parasitoid at 50% of the monitored species (Baxendale *et al* ., 1983).

Other species of Platygasteridae have a similar parasitism of *P. demades* on OLM *D. oleae* . A study on many species of endo-parasitoids belonging to Platygasteridae, parasitizing the larvae of blueberry gall midge *Dasineura oxycoccana* (Johnson), showed that the parasitism has occurred on 2nd and 3rd larval stage of the midge and parasitism was significantly higher in 3rd instars than 2nd instars (Roubos & Liburd, 2013). *D. oxycoccana* larvae spin a silken cocoon within which they pupate. This occurs within the damaged tip except for the final generation each year, which pupates in the soil

where they overwinter. Adults of *D. oxycoccana* & the parasites emerge in the spring (Collins and Eyre, 2010). *Dasineura oxycoccana* larvae dissected from leaf buds contained parasitoid immatures: oblong ovoid eggs visible within the host midgut and cyclopoid larva visible in the host hemocoel. Parasitism rates ranged from 25 to 40%.The majority of parasitized *D. oxycoccana* larvae 62.2 % were parasitized only once. Some midge larvae contained up to seven parasitoid immatures. It could not be determined if the immatures deposited in a given midge larva came from one or multiple parasitoid females(Roubos & Liburd, 2013).

3.2. Percent presence of different endo- parasitoid stages in its population during diapause host phase within field samples during 2013 & 2014

Figs. (4&5) showed that the presence of endo-parasitoid *P. demades* was in juvenile larval stage all over July to November was 80.9 - 100% and 83-100% in its population in 2013 & 2014, respectively. The anatomic examination indicated that the endo-parasitoid was present in this stage along the diapause of its host in the 2nd stage i.e. from July to December. The 2nd stage of midge ranged between 81.36% in October and 100% in August and September of the year 2013, while it ranged between 72.7 % in October to 100% in August and September 2014 (Fig. 2&3).

3.3. Percent presence of different endo- parasitoid stages in its population during activity host phase within field samples during 2013 & 2014

Percent larvae *P. demades* decreases in January as of the active period of its host. The pupae of parasite began to appear during January to June for both study years. High percentage of endo- parasitoid pupae was registered, which reached 83.8 and 69.7% in January and February, respectively in 2013, while it was 75.7 and 67% in the same months respectively, in 2014 (Figs. 4&5). The existence of *P. demades* pupae is accompanied with the 3rd stage & the pupae of olive leaf midge underlying that the endo-parasitoid had left the diapause as its host. The existence of the 3rd stage & the pupae of olive leaf midge was registered during January to June . The highest percentage of the 3rd stage of midge was 43% in January & February in 2013 versus 44.7% in January 2014. While, the highest percentage of midge pupae was 27 and 45.29% in February for the years 2013 & 2014, respectively (Figs. 2 & 3).

P. demades adults began to emerge late February, but they are present during February to end June. High percentages of endo- parasitoid adults in its population were registered during the above-mentioned period, i.e. 77.9 & 65.5% in April for 2013 & 2014 ,respectively and 40.5 & 57% in May for 2013&2014 , respectively (Fig. 4&5). This situation was associated with new attacks of olive leaf midge in its activity period. The appearance of the adults of *D. oleae* and *P. demades* was noticed in the activity phase within appropriate temperature range, where the mean monthly temperature was between 15 - 22 ^{o}C and the relative humidity was 60-70%.

Low percentage of the adults of *P. demades* in October & November was revealed , i.e. between 7-9 % in its population for 2013, and 6% in November for 2014 (Figs. 4 & 5). The existence of this parasite in this period may be due to the appearance of some host larvae that left theirt diapause and continued their growth, may be according to temperature change in October & November. Mean monthly temperature was 20 ^{o}C which was suitable for larvae reactivity. So, there were adults of midge at 6% in its population (Figs. 2&3).

Another studies on different hosts showed that the adults of *P. demades* began to appear 2-3 days after the appearance of *D. mali* and after 3 weeks of *D. pyri* appearance (Tomkins *et al*.,2000 cited from Todd,1959). Shaw *et al*., 2003 pointed out the synchronization between the appearance of adults of *P. demade*s and activity and beginning of eggs deposition of its host *D. mali*, especially for the first generations.

3.4. Synchronization of biology *P. demades* with that of its host *D. oleae*

The anatomic results showed that OLM stopped its growth at the 2nd larval stage for a period with alternating high temperature in the summer & low temperature in the winter specially from early July to mid-December. The host larvae had been reactivated and the 3rd larval stage appeared as temperature rose in February in the next year (Figs. 2&3). However, the endo- parasitoid was at the larval stage in the infected midge larvae during the diapause midge. *P. demades* larvae remain inside the OLM larvae until midge larvae continued growth. Both last larval stage and the pupae of endo- parasitoid appear at the same time that of the 3rd larval stage of midge. That is the midge larva attacked by *P. demades* stopped its development and failed in pupation, after it had sloughed to the 3rd larval stage, *P. demades* larva feed on all the contents of its host and remained inside until emerging as adult, But the undamaged midge larvae had pupated and adults had emerged.

P. demades is synchronized of its biology with that of its different hosts. Other studies showed that the eggs and larva of *P. demades* live inside the egg and larva of the host *D. mali* at all stages, and the adults emerge from the attacked larvae before they become pupae. Tomkins *et al*.(2000) mentioned that

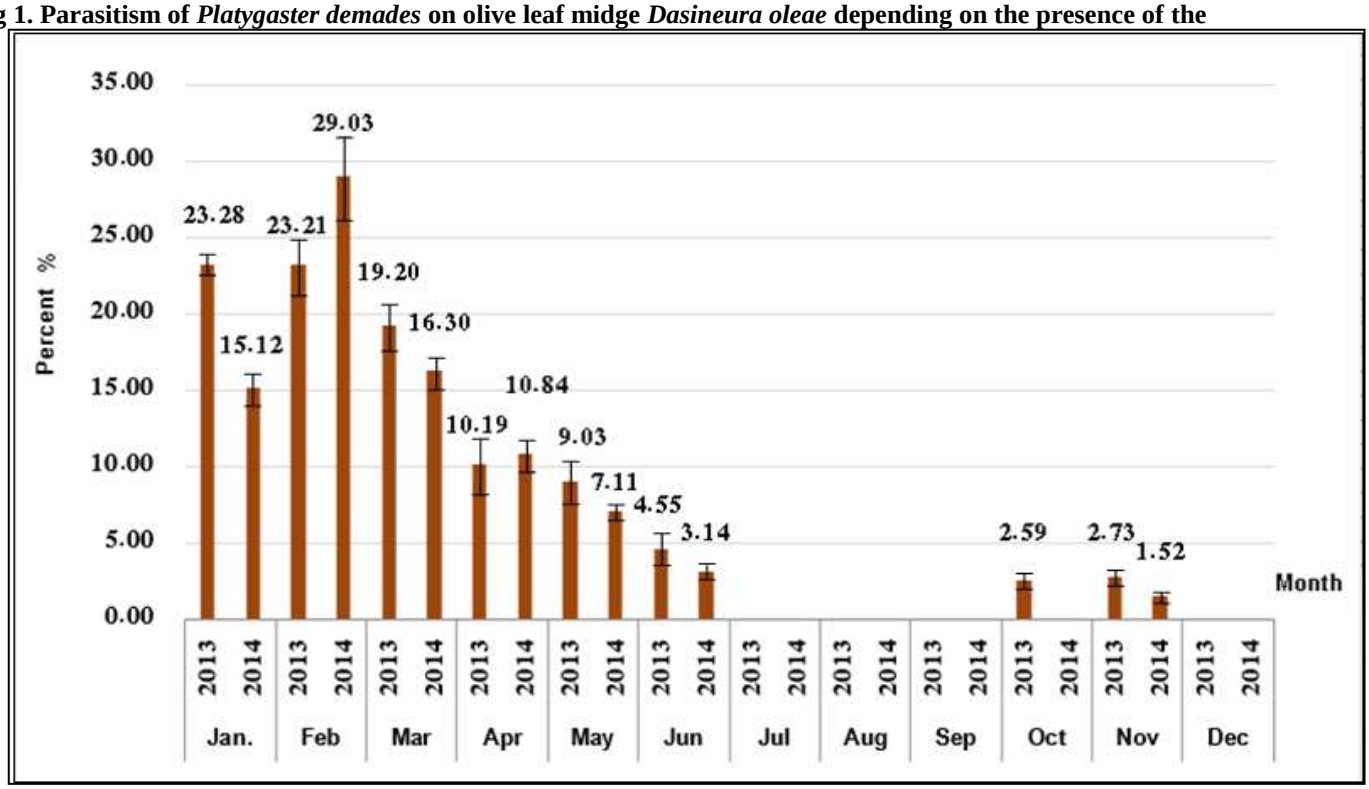
the adults of *P. demades* attacked the eggs of *D. mali* and the embryo develops slowly inside the larva of the host and the adult wasp only emerges before *D. mali* has pupated. Comparing with other species, for example, the adults of *Platygaster chilophagae* have been found where its host found that parasitizes on the eggs or the 1st larval stage of *Chilophaga virgati* . So far, this behavior of parasite never been directly in line with the behavior and biology of its host. However, the parasite *P. chilophagae* could be bred on the last larval stage before the pupation of *Chilophaga virgate* (Johnson *et a*l., 2013).

From the above, it can be concluded that there is a true synchronization between biology of *P. demades* and that of its host *D. oleae*. This phenomenon points out a reciprocal physiological relationship between host & parasite especially for the hormone secretions affecting their growth & development. The reference studies showed that the endo-parasitoids may be to influenced directly or indirectly by its host hormones to continue their growth. This synchronization was obvious for many different parasite species of Hymenoptera & Diptera as the case for the parasite *Opius concolor* from Hymenoptera and its host Mediterranean fruit fly *Ceratitis capitata* so that the parasite continued its development after 36 hours from the injection of Ecdyson hormone(Riddiford,1975).

Also, Synchronization development in the endo-parasitoid *Pseudoperichaeta nigrolineata* Walker (Diptera) and its host *Ostrinia nubilalis* Hubner was noticed, where the endo-parasitoid remain at the 2nd stage as long as the host is in diapause, The tachinid parasitoid larva resumes its growth only at the hosts diapause termination, which was due to the low ecdysterone and also recorded, faster development of the parasitoid larva in non-diapausing host. The relation between parasite growth and hormonal secretions of the host was clear, That the diapause of both host and parasite was broken by the injection with low dose of Ecdyson hormone (Ramadhane *et al* .,1987,1988).

Similar studies by Jadhav & Armes (2013) showed that two larval-pupal tachinid parasitoids; *Goniophthalmus halli* (Mesnil) and *Senometopia* (*Eucarcelia*) *illota* (Curran) were recorded from the pupae of *Helicoverpa armigera* (Hubner). Both the parasitoids enter in diapause following the signals received from their host and continue their development when their host *H. armigera* leaves the diapause.

Fig 1. Parasitism of *Platygaster demades* on olive leaf midge *Dasineura oleae* depending on the presence of the endo-parasitoid pupae & adults in the field samples.



*** Means during 2013 & 2014 are not significantly different at the 5% level.by Student t test (T_2= 0.80 < T_o=1.96)**

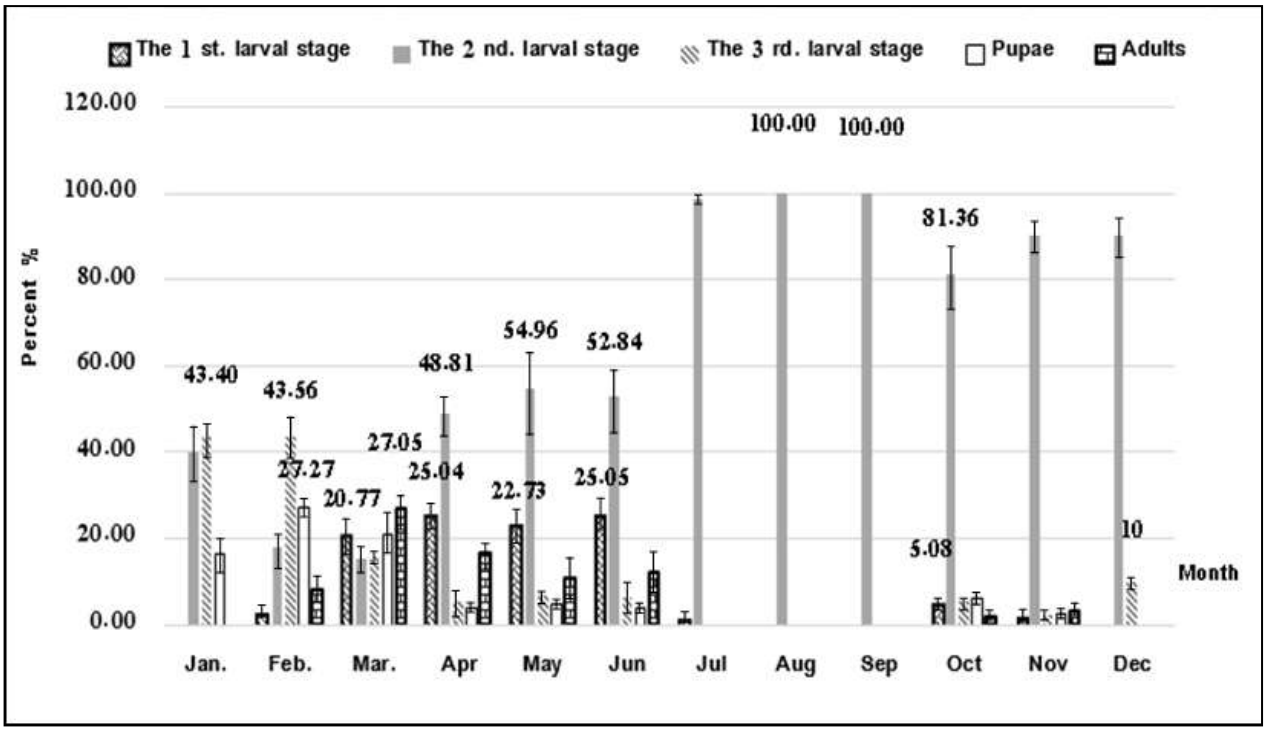


Fig 2. Percentage of different developmental stages of the olive leaf midge in its population on olive fields under natural conditions in the Syrian coast during 2013

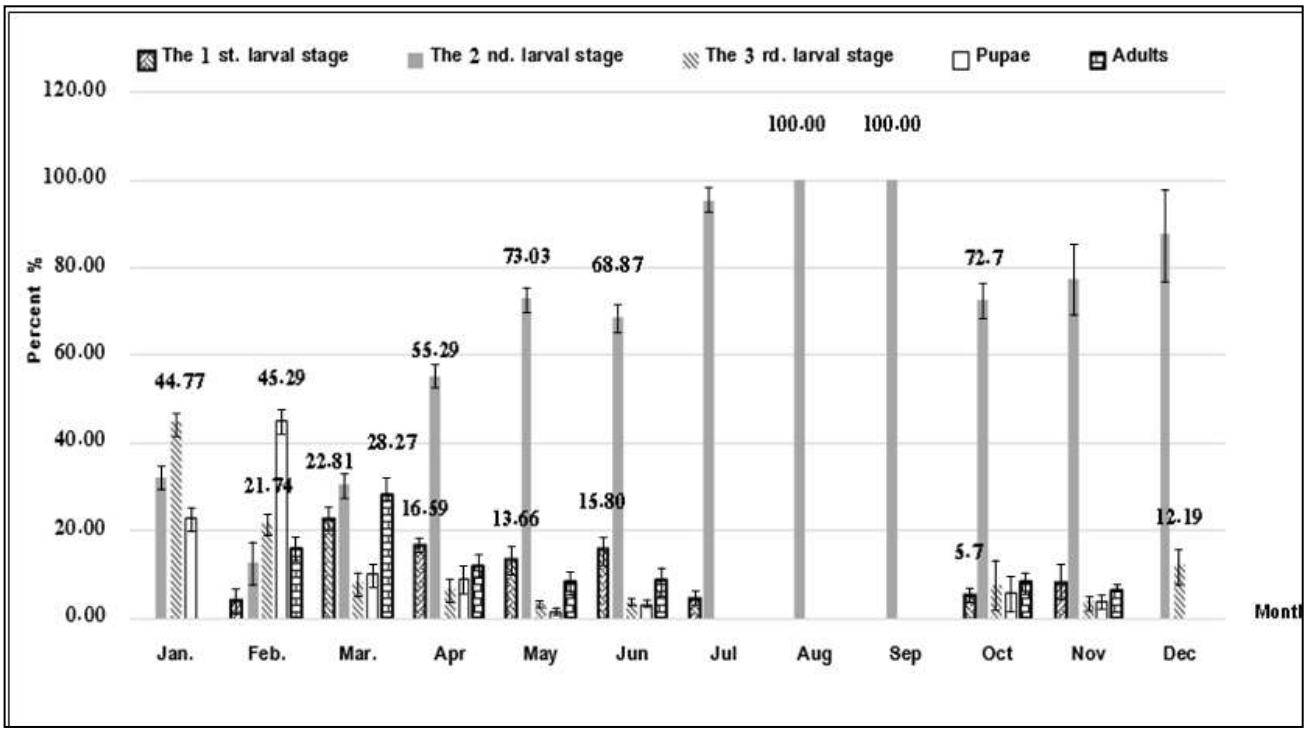


Fig 3. Percentage of different developmental stages of the olive leaf midge in its population on olive fields under natural conditions in the Syrian coast during 2014

*** Means of Fig 2 & Fig 3 during 2013 and 2014 are not significantly different at 5% level for each instar.by Student t test ($T_{1st\ L}$= 0.79 < T_o=1.96, T_{2ndL}=0.92< T_o=1.96, T_{3thL}=0.71< T_o=1.96, T_{Pupae}=0.79< T_o=1.96, T_{Adults}=0.85< T_o=1.96)**

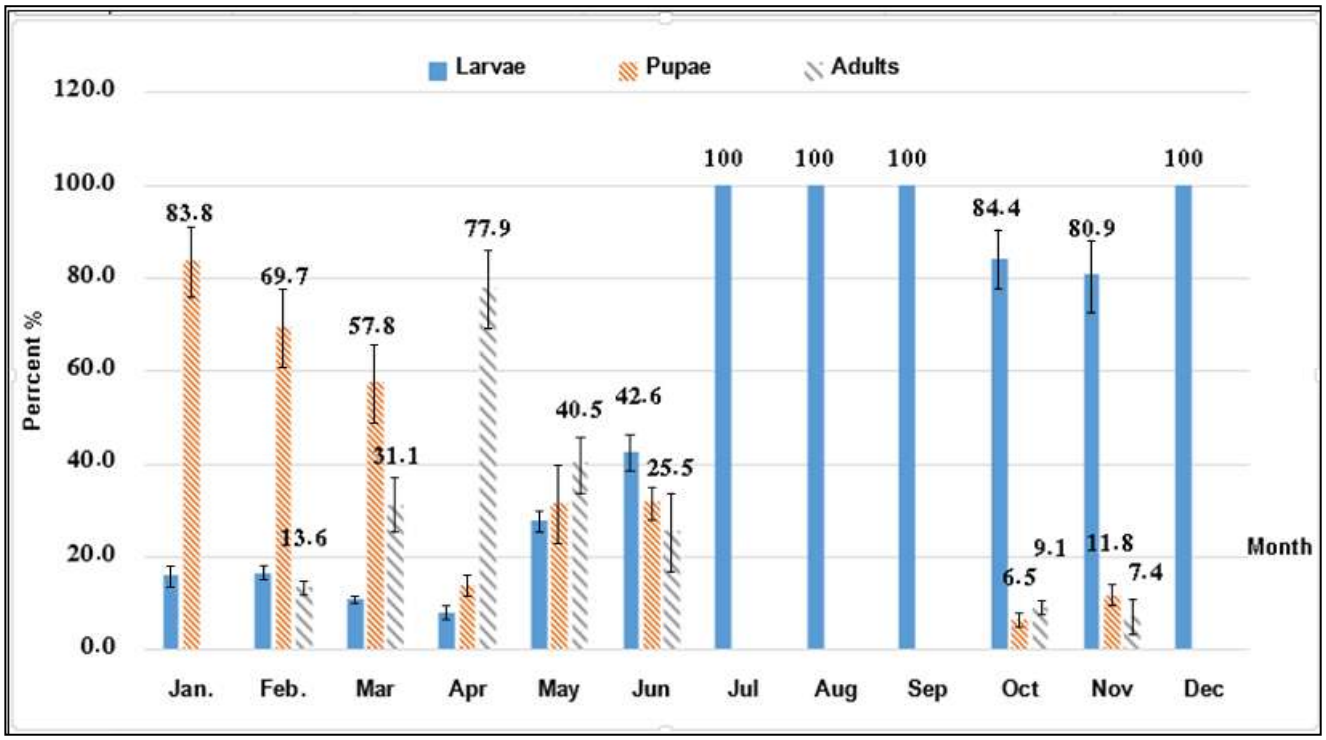


Fig 4. Percentage of different developmental stages of *P.demades* within its population on the host *D.oleae* under natural conditions in Syrian coast during 2013.

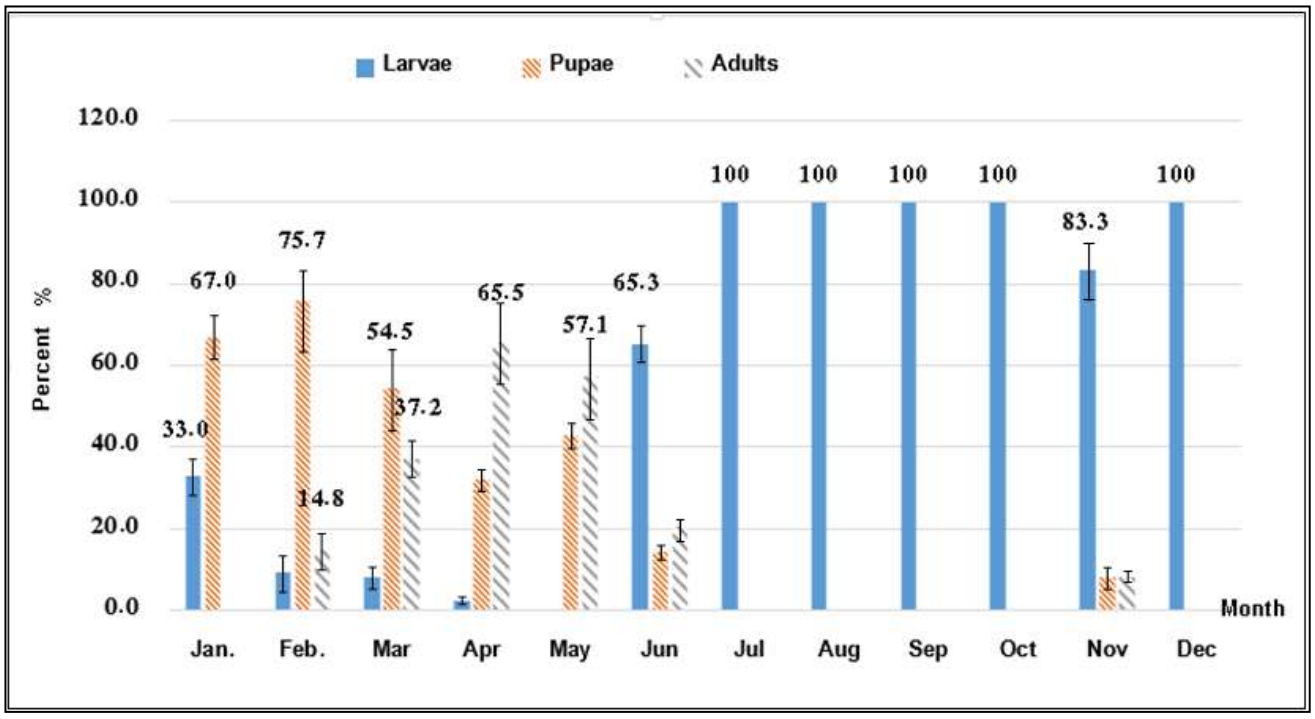


Fig 5. Percentage of different developmental stages of *P.demades* within its population on the host *D.oleae* under natural conditions in Syrian coast during 2014.

*** Means of Fig 4 & Fig 5 during 2013 and 2014 are not significantly different at 5% level for each instar.by Student t test (T_{Larvae}= 0.95 < T_o=1.96, T_{Pupae}=0.95< T_o=1.96, T_{Adults}=0.99< T_o=1.96)**

Finally, the followings can be recommended :

1. A detailed study on synchronization for increasing the efficiency of the endo-parasitoid *P. demades* as the synchronization between biology of *P. demade*s and that of *D. oleae* increases the importance of the endo- parasitoid for pest control.
2. A study of the diapause of the host & endo-parasitoid under certain laboratory conditions at fixed temperatures

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